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A new species of *Physarum* (*Myxomycetes*) from a boreal pine forest in Thuringia (Germany)

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Abstract – A new species of plasmodial slime mold, *Physarum parvicalcareum*, recorded from flowering stalks, leaves and stems of the common heather (*Calluna vulgaris*) that commonly inhabits boreal pine forests in Thuringia (Germany), is described based on morphological features of the capillitium and spores. Phylogenetic trees, reconstructed using data from elongation factor-1 alpha and small subunit ribosomal RNA gene sequence analyses, corroborate the taxonomic status of this new species. In addition, these results support the congruence of morphological and molecular data in this group of eukaryotic microorganisms.

Keywords – molecular phylogenetics, myxomycetes, species concepts

Introduction

Plasmodial slime molds (*Mycetozoa*, commonly referred to as myxomycetes) have been regarded as either "plant-like animals" or "animal-like plants," depending on whether a zoologist or a botanist investigated them. In the 19th century, taxonomists classified the myxomycetes as either fungi or protozoans (Martin & Alexopoulos 1969). However, a detailed analysis of DNA sequence data has recently shown beyond any doubt that these inhabitants of soil and other habitats containing moist, decomposing organic matter comprise a sister taxon to the *Amoebozoa* and hence are members of the Kingdom *Protoctista* (Pawlowski & Burki 2009, Hoppe & Kutschera 2010).

Physarum is the most widely known genus among the myxomycetes, due to the fact that the species *P. polycephalum* serves as a model organism for cell research.

Several years ago, Müller (2007) collected an unidentifiable myxomycete in boreal forests in the Federal State of Thuringia (Germany). Based on morphological, ultrastructural, and molecular data, we herein describe this taxon as a new species of the genus *Physarum*.

Materials and methods

During field trips in 2005 and two subsequent years to the boreal pine (*Pinus sylvestris* L.) forests in the Federal State of Thuringia (close to the towns of Rudolstadt and Mörla in eastern Germany, Central Europe), samples were collected from the leaves, stems, and flowering stalks of the common heather, *Calluna vulgaris* (L.) Hull. This cut plant material was analyzed in the laboratory, using a stereo light microscope (Photomikroskop III, Carl Zeiss, Germany) and a scanning electron microscope (REM, S-4000, Hitachi, Japan) as described by Hoppe & Kutschera (2010). Sample preparations and photographic documentation of the results were carried out as described in the reference cited above. Collections are conserved in Botanische Staatssammlung München (M) and the private collections of T. Hoppe (Germany), H. Müller (Germany), M. Meyer (France), and W. Nowotny (Austria).

Extraction of total deoxynucleic acids (DNA), DNA-amplification via polymerase chain reaction (PCR), and phylogenetic analyses were performed as described in Hoppe & Kutschera (2010). In brief, fruiting bodies were mechanically crushed, after which the homogenized samples were first treated using a FastRNA Pro Red Kit (Solon, Colorado, USA) and then incubated for 90 sec. with the Fast Prep-System FP120 (MP Biomedical) (Costa et al. 2004). In the next step, the samples were incubated for 24 h in a solution of lysozyme (5%, 35°C) and thereafter for 24 to 48 h in proteinase K (5%, 55°C) (Roth, Karlsruhe, Germany). DNA-purifications were performed using a QIAamp DNA Mini Kit (Quiagen, Hilden, Germany). The purified DNA samples were amplified with primers designed for specific elongation factor-1alpha gene sequences (Hoppe & Kutschera 2010) and primers for the small subunit of a ribosomal RNA gene (Kamono & Fukui 2006). The products were purified using NucleoSpin Extract II (Machery-Nagel, Germany) and sequenced. Phylogenetic trees, based on maximum parsimony analyses, were reconstructed as described by Hoppe & Kutschera (2010).

Taxonomy

Physarum parvicalcareum Thom. Hoppe, Holg. Müll. & Kutschera, **sp. nov.**

MycoBank MB 516617; NCBI (GenBank) FJ 558512 and GU 289193 Figs. 1–4

Sporocarpia sessilia, singula vel gregaria vel seria, globosa vel semiglobosa vel brevia plasmodiocarpia, violaceus usque ad aeneus iridescens, (0.3–)0.6–0.8 mm in diametro, usque ad 2 mm longae. Hypothallus membranaceus, translucidus. Peridium simplex, calce non incrustatum, violaceus usque ad aeneus, nonnullum clarum zonatum iridescens, lucem orientem versus visae incoloratum, dehiscentia irregularis. Capillitium reticulatum, album vel alutaceum, lucem orientem versus visae incolor, capilloides vel fasciatum, (1–)2–5(–7) µm in diametro, cum nodis calcareis parvis. Columella vel Pseudocolumella nulla. Sporae frequentes brunneus, lucem orientem versus visae cineraceo-brunneae vel brunneus, globosae, dense cum obscurus, irregulariter verrucosae, 10–11(–12) µm in diametro. Plasmodium ignotum.

Type specimens: Germany, close to Mörla, 50.43°N 11.20°E, on stems, green leaves and flowering stalks of *Calluna vulgaris* in a pine forest, 10 Oct. 2005, Holger Müller. (**Holotype**: Botanische Staatssammlung München (Germany), M 0151322; **Isotype**: private collection of H. Müller (Germany), Müll. 2238).

Etymology: from the Latin *parvus* = small; *calcareus* = calcareous.

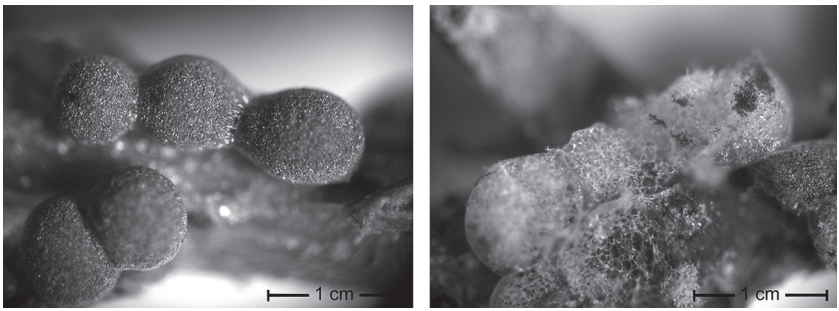

Fig. 1. Photographs of the sporocarps of *Physarum parvicalcareum* attached to a flowering stalk of *Calluna vulgaris*. A- Sporocarps with peridium still intact and spores present. B- Sporocarps after the release of the spores.

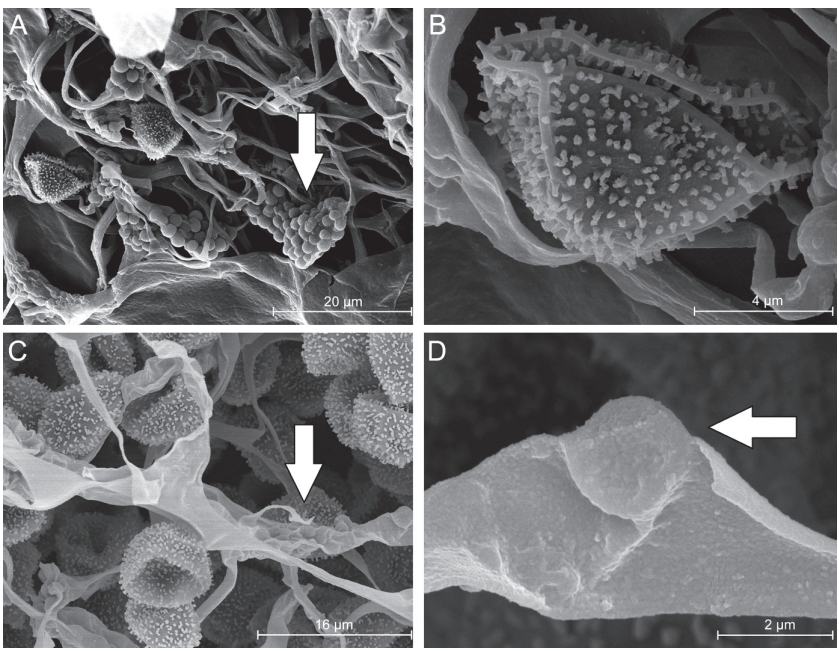

Fig. 2. Scanning electron micrographs of capillitium and spores of *Physarum parvicalcareum*. A- Capillitium with calcareous deposits (arrows). B- Single spore. C- Spores with capillitium that is free of deposits. D- Isolated portion of smooth capillitium with grain (calcareous deposit).

Sporocarps sessile, single, in groups or in lines, globose or sub-globose or short plasmodiocarps, violet to bronze, iridescence in white light, (0.3–)0.6–0.8 mm in diameter, up to 2 mm long (Fig. 1A,B). Hypothallus membranous, transparent, continuous within a group of fruiting bodies. Peridium single,

Table 1: Morphological features of *Physarum parvicalcareum*, *P. nudum,* and *P. cinereum*, based on newly collected and herbarium materials

Character	*P. parvicalcareum*	*P. nudum*	*P. cinereum*
Mature fruiting body	sporocarp or plasmodiocarp	sporocarp or plasmodiocarp	sporocarp or plasmodiocarp
– colour	violet to bronze	white to faint violet	white
– diameter (mm)	0.6–0.8	0.4–1.0	0.3–0.6
– length (mm)	up to 2.0	up to 1.2	up to 0.8
Capillitium surface	smooth or rough	rugged	rugged
– pili length (nm)	up to 300	up to 300	up to 250
Calcareous deposits within fruiting bodies	greatly reduced or absent	present	present

limeless, violet to bronze, some individuals conspicuously iridescent, colourless in transmitted light, dehiscence irregular. Columella or pseudo-columella absent. Capillitium consists of a three-dimensional net with small meshes, white to pale-yellow, colourless in transmitted light, filamentous, with few swellings, covered with small lime granules (Fig. 2A–D), sometimes with a band-like widened appearance, (1–)2–5(–7) µm in diameter. Spores dark-brown, grey-brown or dark-brown in transmitted light, globose, 10–11(–12) µm in diam., densely covered with dark, coarse, more or less irregular warts. Plasmodium not observed.

Ecology and habitat – Living stems, leaves, and flowering stalks of *Calluna vulgaris*; no fruiting bodies were found on nearby plants in the same area.

Expanded description – A phylogenetic analysis, based on novel elongation factor-1 alpha gene sequences supplemented by published data, is depicted in Fig. 3. In addition, a partial (123 bp) sequence of the small subunit of a ribosomal RNA gene was investigated (GenBank-Numbers provided above the lines in Figs. 3, 4) and aligned with morphologically similar species. These data show that *Physarum parvicalcareum* is closely related to the species *P. cinereum* and *P. nudum*, but differs from these taxa in several morphological features (Table 1). Hence, our evolutionary trees (Figs. 3, 4), in tandem with our morphological data (Figs. 1, 2) document *P. parvicalcareum* as a new species and not a morphological variant (variety) of *P. nudum* or one of the other taxa that were analyzed as part of the present study.

Additional specimens examined: **Germany**, close to Mörla, 50.43°N 11.20°E, on stems, green leaves and flowering stalks of *Calluna vulgaris* in a pine forest, 15 Oct. 2005, Holger Müller (Distributed among private collections of H. Müller (Germany: Müll. 2632); M. Meyer (France: 29759, 29760, 30078, 30077); T. Hoppe (Germany: Myx 90); and W. Nowotny (Austria: Now. 13507).

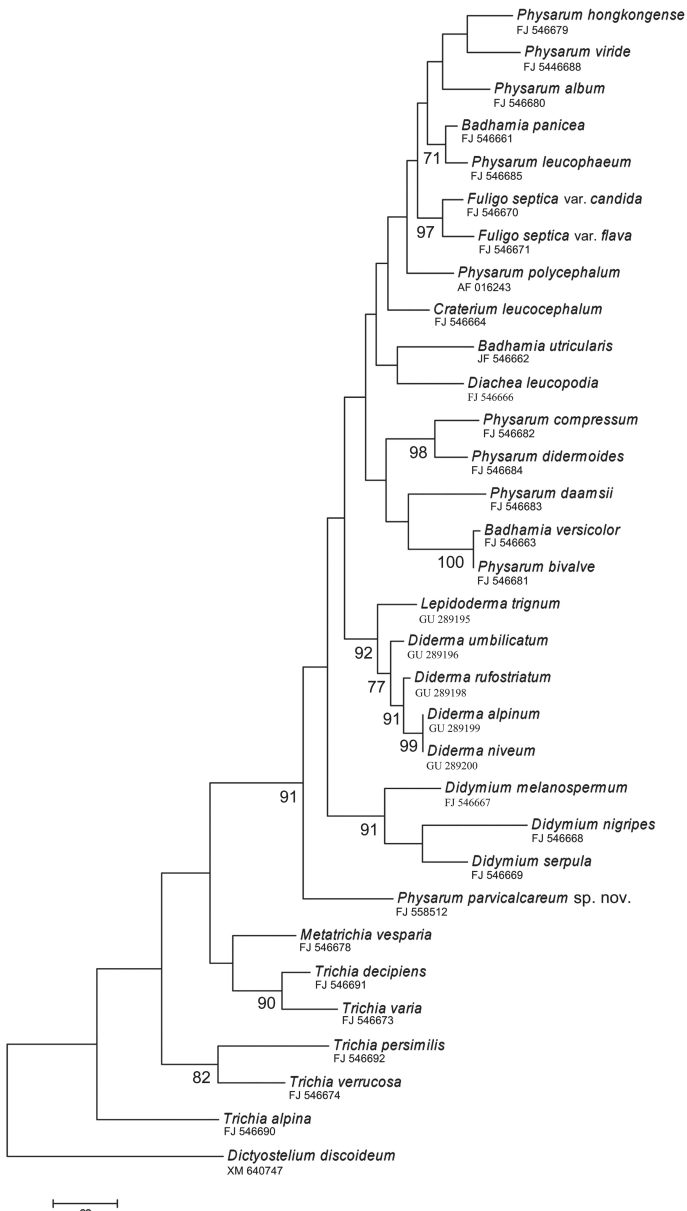

Fig. 3. Phylogenetic tree based on elongation factor-1 alpha gene sequences of 31 myxomycetes, with *Dictyostelium discoideum* as outgroup. The bootstrap values of this maximum parsimony analysis and the GenBank accession numbers are included.

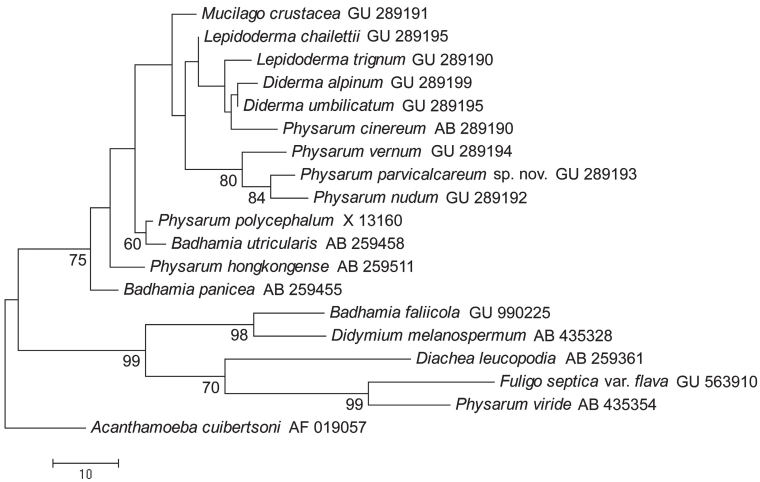

Fig. 4. Phylogenetic tree based on a fragment (123 bp) of the small subunit of a ribosomal RNA gene of 18 morphologically distinct species, with *Acanthamoeba cuibertsoni* as outgroup. The bootstrap values of this maximum parsimony analysis and the GenBank accession numbers are included.

Comments – As pointed out by Neubert et al. (1993, 1995, 2000) and Clark (2000, 2004), myxomycete taxonomy is currently based on the classical morphological species concept. Hence, myxomycetologists have described numerous taxa ("species") that were found in a single habitat or a restricted area, often in low numbers, or even based on a single individual (Lado 2001).

As a result of his survey of recent biosystematic studies in the myxomycetes, Clark (2004) suggested that many commonly accepted morphospecies might, in reality, be species complexes. Moreover, the author suggested that many of the accepted morphospecies might represent morphological variants of one and the same "true" species and therefore should be assigned to the same taxon. In conclusion, Clark (2004) recommended that new species be described only on the basis of numerous collected specimens from different localities so that the problems outlined above could be circumvented.

In this report we describe a new species of myxomycete that was assigned to the genus *Physarum*. The question to be discussed here is whether or not we have met the standards proposed by Clark (2004). In other words, is *P. parvicalcareum* (Fig. 1, 2) only a "variant" of a closely related taxon or does it in fact represent a truly new species?

Our arguments in support of the second conclusion can be summarized as follows. First, the requirement that a new species should be based on an

extensive collection of individuals made on different occasions and (if possible) localities has been met. As documented in a previous report (Müller 2007), numerous samples were collected on several different occasions in Thuringia, and over the past year we found additional specimens of *P. parvicalcareum* in forests in close proximity to the type locality described above (unpublished observations). Second, there is sufficient morphological evidence to separate our new species from all other taxa assigned to the genus *Physarum*, particularly the most closely related species (Table 1). Finally, we analyzed the phylogenetic relationships among 30 myxomycete species within the genera *Trichia*, *Hemitrichia,* and *Metatrichia* in the order *Trichiales* and within the genera *Badhamia*, *Fuligo*, *Craterium*, *Diachea*, *Didymium,* and *Physarum* in the order *Physarales*. These analyses were based on elongation factor-1 alpha gene sequences. In addition, 14 relevant species of the *Physarales* belonging to the genera *Badhamia*, *Diachea*, *Didymium*, *Lepidoderma*, *Physarum* (8 different species), and *Mucilago*, based on a partial sequence of the small subunit of a ribosomal RNA gene, were also investigated. Our quantitative maximum parsimony analyses (Figs. 3, 4) led to the conclusion that *P. parvicalcareum* represents a distinctly new species and not a "morphological variant" of another taxon assigned to the genus *Physarum*.

In summary, our results document that on the above-ground portions (leaves, stems, and flowering stalks) of the common heather (*Calluna vulgaris*) there occurs a myxomycete species that is described here as *P. parvicalcareum*. However, we do not yet know whether or not this new *Physarum* species inhabits its host organism as a commensal or an endophytic parasite (Stephenson & Studlar 1985). It should be noted that we are currently unaware of any other plant species in the boreal pine forest where our new myxomycete was discovered that is inhabited (or infected) by *P. parvicalcareum*. However, more fieldwork is required to further elucidate the entire habitat of this new plant-associated species of the genus *Physarum*.

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